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Environmental Acidification Impact Detection by Examination of Mature Fish Ovaries

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J.F.Klaverkamp, S.L.Leonhard, and K.E.Marshall

Western Region
Department of Fisheries and Oceans
Winnipeg, Manitoba R3T 2N6

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ENVIRONMENTAL ACIDIFICATION IMPACT DETECTION BY EXAMINATION OF MATURE FISH OVARIES

J.H. McCormick, G.N. Stokes and G.J. Portele

U.S. Environmental Protection Agency,
Environmental Research Laboratory-Duluth,
6201 Congdon Boulevard,
Duluth, Minnesota 55804

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Key words: Acid precipitation; minnow, fathead; bass, smallmouth; gametogenesis; reproduction (biology); pH

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La production d'oeufs et le succès d'éclosion de têtes-de-boule au pH de 5.3 varient de faibles à non existants. Des oeufs, pondus et incubés jusqu'à éclosion à des pH de 8.1 et 6.3 produisent 39.7 et 5.7% de larves normales respectivement; les oeufs pondus à un pH de 6.3 et incubés à 5.3 produisent 0.5% de larves normales. L'histologie ovarienne aurait permis de prédire l'insuccès de la reproduction au pH de 5.3. A ce pH, les ovaires de têtes-de-boule contenaient des proportions exceptionnellement élevées de corpora atretica préovulatoires par rapport aux autres stades ovocytaires. Ces données ont été recueillies comme base d'extrapolation pour estimer, par examen de leur condition ovarienne, la dégradation de la reproduction chez des achigans à petite bouche habitant des milieux acides.

Problem Identification

Considerable information is available from both laboratory and field investigations on the levels of pH which cause adverse effects in a number of fishes. Among these are the laboratory studies of Mount (1973) with the fathead minnow and Ruby et al. (1977) with the flagfish; and field studies by Beamish (1976), Beamish and Harvey (1972), and Kennedy (1980), whose paper follows.

Environmental modeling using presently available data will provide information on the trends and expected rates of development of the acidification problem. Past work should indicate at what pH adverse effects can be expected. The work we are involved in is an attempt to provide earlier warning evidence of actual effects, should it be required, to support the need for corrective action.

Approach Rationale

The study area chosen for this work was northeastern Minnesota because of its vulnerability to degradation from acid precipitation. The smallmouth bass was selected as the study species as it has been reported among the most acid sensitive of the area's indigenous fishes (Beamish 1976). Gametogenesis was selected for study because it was reported as probably the most sensitive life stage affected by acid exposure (Beamish 1974; Kennedy 1980, in the following article) and its importance is easily recognized. Field surveys for young-of-the-year (y-o-y) fish were rejected because of the difficulty in obtaining dependable negative data. Ovarian condition was examined by a modified form of the the method developed by Ruby et al. (1977).

Experimental Design

The experimental design called for the acquisition of indices of ovarian conditions (during the spawning and early post-spawning season) of mature fathead minnows reared at known pH levels and relating them to the measured reproductive success within those same populations. The purpose of the fathead minnow work was to serve as a basis of extrapolation from which the ovarian condition of field exposed fishes (smallmouth bass) could be used to predict expected reproductive impairment.

Results and Discussion

Smallmouth bass:

Smallmouth bass ovaries were collected and preserved for histological examination from two of the most acid lakes in northeastern Minnesota where smallmouth bass were reported, and from two non-acid (control) lakes with pH values between 7 and 8. Thumb and McDonald lakes were sampled as the acid lakes; pH values at sampling in June were 6.75 and 6.85, respectively. Spring and Dumbbell lakes were chosen as the non-acid lakes, pH 7.40 and 7.10 respectively, in June. One acid and one control lake were sampled within a day of each other in close geographic proximity (Table 1). (The ovaries from these fish are now undergoing histological examination for the establishment of an index of effect.)

Gonadalsomatic indices[1/] of fish from the two lakes at pH values between 7 and 8 and those of fish from the lakes with pH values below 7 were found to be significantly different (t-test, $p<0.05$) (Table 1). Those indices of the fish from the alkaline lakes (pH above 7) indicated a more advanced stage of maturation. However, analysis of variance did not separate the acid and non-acid lake results into two discrete groups ($p>0.05$), suggesting that the gonadal-somatic index is not a dependable index of subsequent reproductive success.

Fathead minnows:

Adult fathead minnows were sampled from populations exposed to three levels of acidification in artifical stream systems at the U.S. EPA Monticello Ecological Research Station at Monticello, Minnesota. The three exposures were in Mississippi River water: one unaltered, at pH 8.1; and two exposures in H_2SO_4 acidified water, one at pH 6.3 and the other at pH 5.3. Samples were taken four times during the summer of 1979 to determine both their gonadalsomatic indices (Table 2) and the histological condition of their ovaries (Table 3) as related to the progress at the spawning season and the pH of the environment.

Qualitative examination of the ovaries from fish in the artificial streams at the time of first spawning showed that the fish at pH 8.1 were in an advanced stage of developing maturation as much as a week earlier than those at pH 6.3 and pH 5.3 (Hermanutz, pers. comm. 1979). The same stage was, however, eventually reached at pH 6.3 and apparently occasionally at pH 5.3 since spawning, though very limited at pH 5.3, did occur at these lower pH values. Comparisons of the gonadalsomatic indices have not revealed statistically significant differences associated with these three levels of pH ($p>0.05$). Again the gonadalsomatic index does not show promise as a prospective indicator of subsequent reproductive success. Histological indices of gametogenesis are partially completed at this time and the values thus far obtained are presented in Table 3 to be discussed later.

Reproductive impairment as indicated by hatching success of eggs from these same populations has revealed significantly adverse effects (ANOVA, $p<0.05$) when incubated in the artifical streams at pH 6.3 or 5.3 (Fig. 1). Eggs incubated at pH 5.3 were derived from the population at pH 6.3 since no eggs from the fish at pH 5.3 were available. (Eggs from the fish at pH 5.3 were too few to study at any time and none were available at the time of the incubation study.) Incubation studies were performed using 4.6 cm x 4.6 cm stainless steel screen incubation chambers with 2.0 cm deep glass bottoms. The incubating chambers were suspended on styrofoam floats resting in the artifical streams and anchored to the bottom. The chambers were tended daily for removal of dead eggs, and counting dead, deformed and normal larvae. Planaria present at these times were also removed to limit their influence on hatching success of the eggs.

[1/] Gonadalsomatic Index = [ovary weight (g)/total fish weight (g)]x100.

Data from reciprocal transfer of fathead minnow eggs between acid and unacidified stream conditions indicated that parental exposure at pH 6.3 was unimportant in reducing the hatching success of eggs incubated at pH 8.1 (Table 4). Two-way ANOVA found a highly significant effect ($p<0.01$) due to incubation pH and no effect due to parental exposure pH or interaction. Thus it was questionable whether adverse effects of acid environment of pH 6.3, which signficantly reduced hatching success, would be revealed by ovarian examinations. It appears that embryonic development and/or initial larval stages of the fathead minnow are more sensitive parameters of acid impact on year class recruitment than is gametogenesis. To measure this parameter our incubation apparatus or that described by Kennedy (1980; in the following article) seems a good choice, the latter particularly where daily attendance is difficult.

Though the gonadalsomatic indices of the fathead minnows (Table 2) showed no relationship to their pH experience, the histological examination of the ovaries of these same fish indicated that near complete spawning failure at pH 5.3 may have been predicted on the basis of the abundance of pre-ovulatory corpora atretica relative to those of the fish at pH 8.1 (Table 3).

The time of sampling can have an important influence on the results obtained (Table 3). It appears that for the determination of the relative volume of pre-ovulatory corpora atritica sampling the time for the most indicative results would be late in the spawning season to early in the post-spawning period. Pre-ovulatory corpora atretica are a normal phenomenon in the ovaries of mature fish during the reproductive period. Henderson (1963) and others have previously documented this, but the extent and stage of development of the pre-ovulatory corpora atretica found in the fathead minnows at pH 5.3 seem to indicate complete to nearly complete failure of ovulation. Henderson (1963) reports 3 to 5% of the maturing ova became atretic in normal brook trout. Possibly an increase of 15-25% or greater above the number in the species of concern at approximately the same stage of maturation in a similar but unacidified body of water should be cause for concern. The 15-25% was chosen as the critical range since it was this range of values in the fathead minnow that was associated with the poor reproductive success found in the fish at pH 5.3 (Table 3 and Fig. 1), and because scant to no production of juveniles occurred in that artifical stream (Hermanutz, pers. comm. 1979).

Conclusion

It is our opinion that the accrual of large numbers of pre-ovulatory corpora atretica among mature fish exposed to acidified environments shows considerable promise as a means of detecting the adverse impact of environmental acidification on fishes, but that more samples of fish from natural populations where effects are already known will be required before it can be offered as a reliable index of reproductive inhibition.

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Table 1. Gonadalsomatic Indices of Northeastern Minnesota Smallmouth Bass in Lakes with Different pH Values.

	Alkaline Lakes		Acid Lakes	
	Spring 6/5-6/79	Dumbbell 6/23-24/79	Thumb 6/5-6/79	McDonald 6/24-26/79[a]
pH[1]	7.4	7.1	6.75	6.85
$\bar{X}$ G.S.I.[2]	7.24 (4.2-14.7)[b]	4.31 (1.2-8.5)	3.59 (1.0-8.3)	2.85 (0.7-4.2)
Sample size	12	10	9	6
[3]				

[1] The pH values reported are those of the lakes at athe time of fish collections.

[2] G.S.I.= Gonadalsomatic Index.

[3] Lines underscore values not found significantly different, Scheffe's test ($p=0.05$).

[a] Sampling dates.

[b] (Range).

Table 2. Fathead Minnow Gonadalsomatic Indices from Acid Treated and Untreated Mississippi River Water Artifical Streams

	pH		
Sampling date	8.1	6.3	5.3
6/19	18.1 (13.2-22.6)	17.4 (11.0-26.0)	15.4 (7.5-28.6)
7/12	12.1 (2.1-17.0)	12.5 (2.1-19.3)	16.6 (6.8-25.8)
8/14 [a]	2.7 (0.5-11.4)	8.6 (1.7-13.2)	5.6 (2.3-8.4)

[a] Fourth sample not yet processed.

Table 3. Mean percent of Fathead Minnow Ovary Oocyte Volume Occupied by Pre-Ovulatory Corpora Atretica When Exposed to Three Levels of pH (fifty oocytes were examined per fish; 5 fish/treatment and date).

Sampling dates	Pre-ovulatory corpora atretica		
	pH 8.0	pH 6.3	pH 5.3
6/19/79	0.0	5.2**	4.0
7/12/79	0.0	7.6	19.6*
8/14-15/79	2.8	22.4	54.0**

*, ** Significantly different (p=0.05, and p=0.01, respectively) Dunnett's one-tailed test using arc sine transformed percent data.

Table 4. Hatching Success, (%) Normal Larvae Produced from Fathead Minnow Eggs When Incubated at Two pH Levels and Influenced by Previous pH Experience (50 eggs/test lot).

Previous pH experience (Gametogenesis-early cleavage)	Egg lots	Replicates	Incubation pH 8.1	pH 6.3
8.1	V	A	56	0
		B	26	64[a]
	VI	A	26	6
		B	36	6
		$\bar{X}$	35	4
6.3	VII	A	48	2
		B	40	4
	VIII	A	30	0
		B	30	4
		$\bar{X}$	37	3

[a] This value is a statistical outlier ($p<0.99$) and not used in the calculated mean reported below. Two-way ANOV using arc sine transfered % data including the outlier for the most conservative test, found a highly signifiant incubation pH effect and no effect due to parental pH exposure or interaction.

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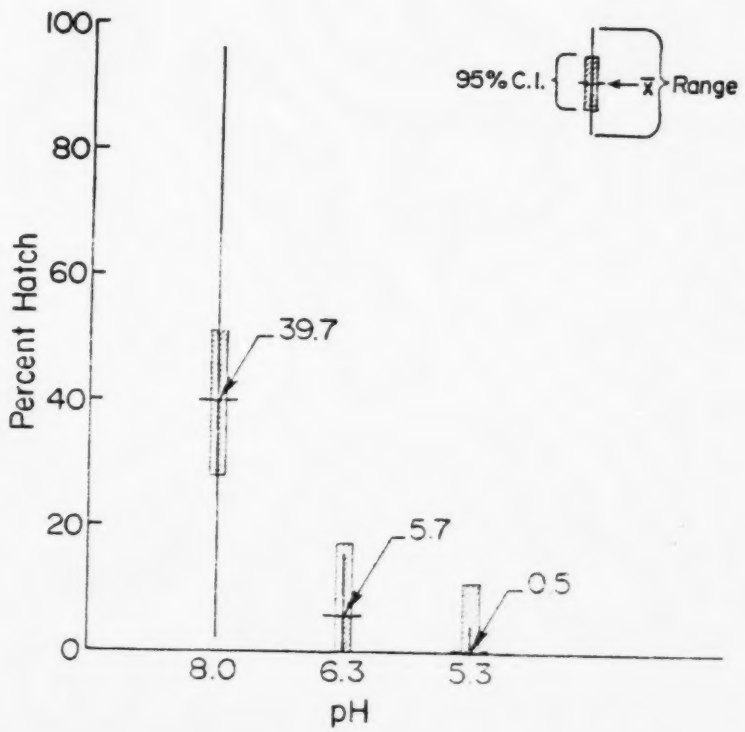

Fig. 1. Percent normal larvae hatched from fathead minnow eggs incubated at different pH's.